EPA/635/R-00/006

TOXICOLOGICAL REVIEW

OF

CHLORAL HYDRATE

(CAS No. 302-17-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

August 2000

U.S. Environmental Protection Agency
Washington, DC

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to chloral hydrate. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of chloral hydrate.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 202-566-1676.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agencywide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for chloral hydrate has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (1996a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), and *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in*

Health Risk Assessment (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Chloral hydrate is not known to occur as a natural product. For the general public, the major route of exposure to chloral hydrate is from drinking water. Chloral hydrate and its metabolites, trichloroacetic acid and dichloroacetic acid, are formed as by-products when water is disinfected with chlorine. The typical concentration in a public water supply in the United States is 5 µg/L (U.S. EPA, 1994d). Additional chloral hydrate can be formed if water containing chlorine is mixed with food containing humic and fulvic acids (Wu et al., 1998). The low volatility of chloral hydrate from a water solution precludes significant exposure by inhalation. Chloral hydrate is also a metabolite of trichloroethylene and tetrachloroethylene. Humans will be exposed to chloral hydrate if they are exposed to these chemicals. Chloral hydrate is currently approved by FDA as a habit-forming, central nervous system depressant (Schedule IV, 21 CFR §329.1 and §1308.14) for use in adult and pediatric medicine.

Chloral (CAS # 75-87-6) is the anhydrous form of the chemical. Chloral is used as an intermediate in the synthesis of the insecticides DDT, methoxychlor, naled, trichlorfon, and dichlorvos, and the herbicide trichloracetic acid (IARC, 1995). The conversion from chloral to chloral hydrate occurs spontaneously when chloral is placed in an aqueous media.

Chloral hydrate could be released to the environment from wastewater treatment facilities, from the manufacture of pharmaceutical-grade chloral hydrate, and from the waste stream during the manufacture of insecticides and herbicides that use chloral as an intermediate.

Chemical and physical properties of chloral hydrate are presented below (IARC, 1995).

CAS Name	2,2,2-trichloro-1,1-ethanediol
IUPAC Name	Chloral hydrate
Primary Synonyms	Chloral monohydrate Trichloroacetaldehyde hydrate

	Trichloroacetaldehyde monohydrate 1,1,1-trichloro-2,2-dihydroxyethane Noctec (formulary name)
CAS Number	302-17-0
Chemical Formula	CCl_3- $CH(OH)_2$
Molecular Weight	165.42
Boiling Point	96-98 °C (decomposes)
Melting Point	57 °C
Specific Gravity	1.908 (20 °C)
Vapor Pressure	15 mm Hg (25 °C)
Solubility	soluble in water, acetone, benzene, chloroform, diethylether, ethanol, and methyl ethyl ketone
Octanol/Water Partition Coefficient (log K_{ow})	0.99

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

Chloral hydrate is completely absorbed following oral administration. Qualitatively similar metabolism occurs in mice, rats, dogs, Japanese Medaka, and humans (Abbas et al., 1996; Abbas and Fisher, 1997; Beland et al., 1998; Breimer, 1977; Elfarra et al., 1998; Fisher et al., 1998; Goodman and Gilman, 1985; Gorecki et al., 1990; Gosselin et al., 1981; Greenberg et al., 1999; Henderson et al., 1997; Hindmarsh et al., 1991; Hobara et al., 1986, 1987a,b, 1988a,b; Lipscomb et al., 1996, 1998; Marshall and Owens, 1954; Mayers et al., 1991; Merdink et al., 1998, 1999; Owens and Marshall, 1955; Reimche et al., 1989; Stenner et al., 1997, 1998). The metabolic pathway is shown in Figure 1.

Chloral hydrate is rapidly metabolized in both hepatic and extrahepatic tissues to trichloroethanol and trichloroacetic acid. The alcohol dehydrogenase responsible for reducing it to trichloroethanol is located in both liver and erythrocytes. A portion of the trichloroethanol produced is conjugated with glucuronic acid to form trichloroethanol-$-glucuronide, which is excreted in the urine. A portion of the trichloroethanol-glucuronide is secreted into the bile and is subject to enterohepatic circulation. Oxidation of chloral hydrate to trichloroacetic acid occurs primarily in the liver and kidney via an aldehyde dehydrogenase using nicotinamide adenine

dinucleotide (NAD) as a cofactor. The major route of excretion of the metabolites of chloral hydrate is the urine.

Chloral hydrate and its metabolites have been found in milk (Bernstine et al., 1956). As soon as lactation started, mothers (n=50) were treated with a 1.33 g rectal suppository of chloral hydrate. Samples of maternal blood and breast milk were taken for analysis from 15 minutes and at varying intervals up to 24 hours following administration of the drug. The maximum concentration of the sum of chloral hydrate, trichloroethanol, and trichloroethanol-glucuronide (the potential pharmacologically active species) in milk occurred within 1 hour after administration of the drug and averaged 53 mg/L (n=11). The amount of chloral hydrate required for sedation in infants is 10 mg in a single feeding of 100 mL of milk.

In mice and rats, 8% of the administered dose of chloral hydrate is directly eliminated in urine, 15% is converted to trichloroacetic acid (including the contribution from enterohepatic circulation), and 77% is converted to trichloroethanol (Beland et al., 1998). In humans 92% of the administered dose of chloral hydrate is converted to trichloroethanol and 8% is converted directly to trichloroacetic acid; additional trichloroacetic acid is formed during enterohepatic circulation of trichloroethanol such that 35% of the initial dose of chloral hydrate is converted to trichloroacetic acid (Allen and Fisher, 1993).

Although earlier reports claimed detection of substantial quantities of dichloroacetic acid in blood from studies in rodents (Abbas et al., 1996), data show that the dichloroacetic acid is most likely formed by an acid-catalyzed dechlorination of trichloroacetic acid in the presence of reduced hemoglobin (Ketcha et al., 1996). Recent experimental data and pharmacokinetic model simulations in rodents suggest that dichloroacetic acid occurs only as a short-lived metabolite in the liver and is rapidly converted to two-carbon, nonchlorinated metabolites and carbon dioxide (Merdink et al., 1998). Using a different extraction procedure less likely to induce the artifactual formation of dichloroacetic acid, Henderson et al. (1997) showed the presence of dichloroacetic acid in children treated with chloral hydrate in a clinic.

Breimer (1977) administered an aqueous solution of chloral hydrate to five human volunteers. Each volunteer received a single oral dose of 15 mg/kg. Chloral hydrate could not be detected in the plasma even at the first sampling time of 10 minutes. A method with a limit of detection of 0.5 mg/L was used. Trichloroethanol and trichloroethanol-glucuronide reached peak concentrations 20 to 60 minutes after administration of chloral hydrate. The maximum concentration of trichloroethanol in the plasma was about 5 mg/L. The average half-lives of trichloroethanol and trichloroethanol-glucuronide were 8 hours (range 7-9.5 hours) and 6.7 hours (range 6-8 hours), respectively. The half-life of trichloroacetic acid was about 4 days.

Zimmermann et al. (1998) administered a single dose of 250 mg chloral hydrate in 150 mL of drinking water to 18 healthy male volunteers (20 to 28 years of age). Chloral hydrate, trichloroethanol, and trichloroacetic acid were measured in plasma. Chloral hydrate could only be detected 8 to 60 minutes after dosing in 15 of 18 plasma samples. The measured

concentration of chloral hydrate in plasma ranged from 0.1 mg/L (the limit of detection) to 1 mg/L. The mean maximum plasma concentration of trichloroethanol of 3 mg/L was achieved 0.67 hours after dosing. The mean maximum plasma concentration of trichloroacetic acid of 8 mg/L was achieved 32 hours after dosing. The terminal half-life for trichloroethanol was 9.3 to 10.2 hours and for trichloroacetic acid was 89 to 94 hours.

Two toxicokinetic models for chloral hydrate in rats and mice are available (Abbas et al., 1996; Beland et al., 1998). Beland et al. (1998) treated rats and mice with chloral hydrate by gavage with 1 or 12 doses using 50 or 200 mg/kg per dose. The maximum concentrations of chloral hydrate, trichloroethanol, and trichloroethanol-glucuronide in the plasma were observed at the initial sampling time of 0.25 hour. The half-life of chloral hydrate in the plasma was approximately 3 minutes. The half-lives of trichloroethanol and trichloroethanol-glucuronide in the mouse plasma were approximately 5 and 7 minutes, respectively. Trichloroacetic acid was the major metabolite found in the mouse plasma, with the maximum concentration being reached 1-6 hours after dosing. The half-life of trichloroacetic acid in the mouse plasma was approximately 8-11 hours. Comparable values were obtained for rats.

Estimates of the concentrations of trichloroacetic acid and trichloroethanol at steady state under various exposure conditions are in Appendix B.

Several studies have investigated the age-dependence of the metabolism of chloral hydrate (Gorecki et al., 1990; Hindmarsh et al., 1991; Mayers et al., 1991; Reimche et al., 1989). These studies were conducted in critically ill patients in neonatal and pediatric intensive care units and may not be representative of a population of healthy infants. The half-lives for trichloroethanol and its glucuronide were increased fourfold in preterm and threefold in full-term infants. The half-life for trichloroethanol in toddlers was similar to that reported for adults. The reported half-lives for elimination of trichloroethanol were 39.8 hours, 27.8 hours, and 9.67 hours for preterm infants, full-term infants, and toddlers, respectively (Mayers et al., 1991), compared to 7-9.5 hours reported by Breimer (1977) and 9.3-10.2 hours reported by Zimmermann et al. (1998). These age-related differences likely are the result of the immaturity of hepatic metabolism, particularly glucuronidation, and decreased glomerular filtration.

Kaplan et al. (1967) investigated the effect of ethanol consumption on the metabolism of chloral hydrate in adults. Subjects ingested doses of ethanol (880 mg/kg), chloral hydrate (9 to 14 mg/kg), or both. In subjects consuming both ethanol and chloral hydrate, the concentration of trichloroethanol in blood rose more rapidly and reached a higher concentration than in subjects consuming chloral hydrate only. Ethanol promotes the formation of trichloroethanol because the oxidation of ethanol provides NADH used for the reduction of chloral hydrate (Watanabe et al., 1998).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS - EPIDEMIOLOGY AND CASE REPORTS

Chloral hydrate has been widely used as a sedative/hypnotic drug in humans. The recommended dose for an adult as a sedative is 250 mg three times a day (equivalent to 10.7 mg/kg-day); the recommended dose as a hypnotic is 500-1,000 mg (equivalent to 7-14 mg/kg) (Goodman and Gilman, 1985). The recommended dose for a child as a sedative is 9 mg/kg, three times a day, to 25 mg/kg in single dose (Hindmarsh et al., 1991). The recommended dose for a child undergoing a medical or dental procedure is 50 to 100 mg/kg (Badalaty et al., 1990; Fox et al., 1990). A child is typically given a higher dose than an adult because a deeper level of sedation is desired to obtain better cooperation from the child during the medical or dental procedure. There is no evidence that a child is less sensitive than an adult to the sedative effects of chloral hydrate. Because of the rapid metabolism of chloral hydrate, trichloroethanol is responsible for the majority of the pharmacological activity (Marshall and Owens, 1954; Breimer, 1977; Goodman and Gilman, 1985). The concentration of trichloroethanol in the plasma in the pharmacologically active range is approximately 5 mg/L and above, and in the toxic range is 100 mg/L and above.

Chloral hydrate is irritating to the skin and mucous membranes and often causes gastric distress (nausea and vomiting) at recommended doses. There are no reports of sensitization in humans. Overdoses produce (in order of progression) ataxia, lethargy, deep coma, respiratory depression, hypotension, and cardiac arrhythmias. The life-threatening effects are from severe respiratory depression, hypotension, and cardiac arrhythmias. For some representative case reports, see Anyebuno and Rosenfeld (1991), Ludwigs et al. (1996), Marshall (1977), and Sing et al. (1996). A potentially life-threatening oral dose for humans is approximately 10 g (143 mg/kg), although death has been reported from as little as 4 g, and some individuals have survived ingesting 30 g or more. Extended abuse of chloral hydrate may result in development of paranoid behavior, in tolerance to the pharmacological effect, and in physical dependence or addiction to chloral hydrate. Sudden withdrawal after habituation can precipitate seizure, delirium, and death in untreated individuals.

Shapiro et al. (1969) reviewed the medical records of 1,618 patients who had received chloral hydrate at 1 g (213 patients, 13%), 0.5 g (1,345 patients, 83%), or various other doses (60 patients, 4%). Adverse reactions were reported in 38 patients (2.3%). Of these patients, 4 received 1 g, 1 received 0.75 g, and 33 received 0.5 g. Reported adverse reactions included gastrointestinal symptoms in 10 patients, depression of the central nervous system in 20 patients, skin rash in 5 patients, prolonged prothrombin time in 1 patient, and bradycardia in 1 patient. In all patients the side effects disappeared when chloral hydrate therapy was stopped. There was no evidence of association between adverse side effects and age, weight, or sex.

Miller and Greenblatt (1979) reviewed medical records of 5,435 hospital patients who received chloral hydrate at a dose of either 0.5 g (about 7 to 8 mg/kg) or 1 g (about 14 to 16

mg/kg). Adverse reactions were noted in 119 cases (2.2%). Central nervous system (CNS) depression was most common (58 patients, or 1.1%), with minor sensitivity reactions, including rash, pruritus, fever, and eosinophilia, second most common (19 patients, or 0.35%). Other adverse reactions included gastrointestinal disturbances (0.28%) and CNS excitement (0.22%). Three individuals (0.05%) were judged to have life-threatening reactions involving CNS depression, asterixis (flapping tremor characterized by an intermittent lapse of assumed posture due to involuntary sustained contractions of groups of muscles), or hypotension. The data show that adverse reactions involving the central nervous system became more frequent with increasing dosage in patients older than 50 years, in patients who died during hospitalization, in patients who concurrently received benzodiazepine antianxiety drugs, and in patients with elevated levels of blood urea nitrogen.

Greenberg et al. (1991) reported various side effects experienced by children receiving chloral hydrate sedation in preparation for computer tomography (CT) procedures. In a "high-dose" group, composed of 295 children (average age 2.18 years) that received a single dose of 80 to 100 mg/kg and a maximum total dose of 2 g, adverse reactions occurred in 23 of the patients (7%) and included vomiting (14 patients), hyperactivity (5 patients), and respiratory symptoms such as wheezing and secretion aspiration (4 patients). Cardiac monitoring did not reveal any abnormalities or arrhythmias in any of the children. A second "lower-dose" cohort of 111 children (average age 1.9 years) received 40 to 75 mg/kg chloral hydrate. These patients received the lower dose because of existing liver or renal impairment, respiratory insufficiency, or CNS depression. There were no adverse side effects or complications reported in this group. Children with severe liver or renal disease or affected by severe CNS depression were not treated with chloral hydrate.

Lambert et al. (1990) conducted a retrospective analysis of hospital medical records to investigate a possible link between chloral hydrate administration and direct hyperbilirubinemia (DHB), an increase in the concentration of unconjugated bilirubin in the serum, in neonates following prolonged administration of chloral hydrate (25 to 50 mg/kg administered for up to 20 days). In the first study, the DHB was of unknown etiology in 10 of the 14 newborns with DHB; all 10 of these DHB patients had received chloral hydrate. In the second study, among 44 newborns who had received chloral hydrate, 10 patients that developed DHB had received a mean cumulative dose of 1,035 mg/kg. In contrast, 34 patients whose direct bilirubin levels were within normal ranges received a mean cumulative dose of 183 mg/kg. As the total bilirubin levels (free plus conjugated bilirubin) were the same in both groups and within the normal range, the increased direct bilirubin could result from competition between trichloroethanol and bilirubin in the glucuronidation pathway, known to function suboptimally in neonates.

Kaplan et al. (1967) investigated whether ethanol ingestion altered the metabolism of chloral hydrate or increased subjective symptoms. Five male volunteers weighing 70 to 107 kg consumed ethanol (880 mg/kg), chloral hydrate (1 gram, 9 to 14 mg/kg), or both. Blood pressure and cardiac rate did not vary significantly among treatments. In the presence of ethanol, the concentration of trichloroethanol in the blood rose more rapidly and reached a higher

concentration, but the rate of depletion was not significantly changed. The increase in the concentration of trichloroethanol was not sufficient to produce a marked enhancement of the hypnotic effect. The volunteers reported symptoms (drowsiness, dizziness, blurred vision) and their severity during the 6-hour observation period. At all time points, the rank order of effects was: ethanol plus chloral hydrate > ethanol > chloral hydrate.

No long-term studies of chloral hydrate in humans were located in the published literature.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS

4.2.1. Oral

Sanders et al. (1982) studied the acute toxicity of chloral hydrate in CD-1 mice. Groups of 8 male and 8 female mice were given chloral hydrate by gavage in distilled water at 300, 600, 900, 1,200, 1,500, or 1,800 mg/kg. No deaths occurred at 900 mg/kg or below in either sex. The calculated LD_{50} for females was 1,265 mg/kg and for males was 1,442 mg/kg. Effects were seen within 10 minutes of dosing. The mice became sedated at 300 mg/kg. At 600 and 900 mg/kg, the animals became lethargic and exhibited loss of righting reflex. Respiration was markedly inhibited at 1,200, 1,500, and 1,800 mg/kg. Inhibition of respiration appeared to be the immediate cause of death. Most deaths occurred within 4 hours at 1,800 mg/kg. At 1,200 and 1,500 mg/kg, some deaths occurred after 4 hours, with all deaths occurring within 24 hours.

Goldenthal (1971) reported LD_{50}s of 479 mg/kg and 285 mg/kg in adult and 1-2 day old Charles River Sprague-Dawley rats, respectively.

Sanders et al. (1982) studied the short-term toxicity of chloral hydrate in mice. Groups of male CD-1 mice were given chloral hydrate by gavage in distilled water at 14.4 or 144 mg/kg-day for 14 days. No significant effect on body weight was observed. No changes in internal organs were noted on gross examination. Groups of 11 to 12 mice were evaluated for several parameters. No significant effects on hematological or serum biochemical parameters were noted. There was a statistically significant ($p<0.05$) increase in liver weight (17%) and a decrease in spleen weight (27%) at 144 mg/kg-day. The NOAEL in this study is 14.4 mg/kg-day; the LOAEL is 144 mg/kg-day.

Sanders et al. (1982) administered chloral hydrate in drinking water to CD-1 mice at 70 or 700 mg/L (equivalent to 16 mg/kg-day or 160 mg/kg-day) for 90 days. In males, hepatomegaly (an increase in weight of 20% and 34% at the low and high exposure, respectively) and microsome proliferation (no increase in total microsomal protein, increase in cytochrome b_5 of 26% and 40%, increase in aminopyrine N-demethylase of 28% and 20%, and increase in aniline hydroxylase of 24% and 30% at the low and high exposure, respectively, when reported

as mg of protein per mg of total liver protein) were observed. There were no biologically significant changes in serum enzymes. Hepatomegaly was not seen in females, but there were changes in hepatic microsomal parameters (increase in total microsomal protein of 10%, increase in aniline hydroxylase of 23%, and decrease in cytochrome b_5 of 12%, when reported as mg of protein per mg of total liver protein) only at the high exposure. No other significant toxicological changes were observed. Based on hepatomegaly and changes in microsomal parameters in males at the high exposure, the LOAEL in this study is 160 mg/kg-day; the NOAEL is 16 mg/kg-day.

Rijhsinghani et al. (1986) evaluated the carcinogenic potential in male C57BL × $C3HF_1$ mice. Groups of 15-day-old mice received a single dose of chloral hydrate by gavage in distilled water at 0, 5, or 10 mg/kg (26, 15, and 14 mice per group, respectively). Animals were sacrificed when moribund or at week 78, week 88, or between weeks 89 and 92. Livers were examined histopathologically using light and electron microscopy. In mice sacrificed 48 to 92 weeks after treatment, the incidence of hepatic nodules (adenomas or trabecular carcinomas) was 3/9 and 6/8 for animals from the 5 and 10 mg/kg-day dose groups, respectively, compared with 2/19 in controls. The increase in tumors was statistically significant ($p<0.05$) only in the 10 mg/kg group.

NTP (2000a) investigated the ability of a single exposure to chloral hydrate to induce tumors in female and male $B6C3F_1$ mice. Groups of 15-day-old or 28-day-old female mice (48 animals per dose group) received a single gavage dose of chloral hydrate in distilled water at 0, 10, 25, or 50 mg/kg. An identical study was conducted in 15-day-old male mice. All animals were sacrificed at 105 weeks of age. No neoplastic or non-neoplastic effects were found in any organ at any exposure.

Daniel et al. (1992a) exposed 40 male $B6C3F_1$ mice for 104 weeks to drinking water containing chloral hydrate at 1 g/L (equivalent to 166 mg/kg-day). Untreated control animals (23 in one group and 10 in a second group) received distilled water. Interim sacrifices were conducted at 30 and 60 weeks of exposure (5 animals per group at each sacrifice interval). Complete necropsy and microscopic examination were performed. There were no significant treatment-related effects on survival or body weight. There were no changes in spleen, kidneys, or testes weights, or histopathological changes in any tissue except the liver. The toxicity in the liver was characterized by increased absolute liver weight and liver-to-body weight ratio at all three sacrifice intervals. At week 104, liver weight was 37% higher than controls, and liver-to-body weight ratio was 42% higher than controls. Hepatocellular necrosis was noted in 10/24 (42%) treated animals; other pathological changes of mild severity reported in the livers of treated animals included cytoplasmic vacuolization, cytomegaly, and cytoplasmic alteration. The prevalence of liver tumors at terminal sacrifice was statistically significantly ($p<0.05$) increased over controls, with hepatocellular carcinomas in 11/24 and hepatocellular adenomas in 7/24; for carcinomas and adenomas combined, the prevalence was 17/24. In control animals, carcinomas, adenomas, and carcinomas and adenomas (combined) occurred in 2/20, 1/20, and 3/20, respectively. At the 60-week sacrifice, there were 2/5 treated animals with hepatocellular

carcinomas, compared with 0/5 controls. No carcinomas, adenomas, or hyperplastic nodules were reported in animals sacrificed at week 30.

George et al. (2000) conducted a chronic bioassay for carcinogenicity in male $B6C3F_1$ mice. Mice were administered chloral hydrate in drinking water for 104 weeks. Mice (72 in each group) had a mean exposure of 0, 13.5, 65, or 146.6 mg/kg-day. At the termination of the study, a complete necropsy and histopathological examination of liver, kidney, spleen, and testes from all animals was conducted. In addition a complete histopathological examination was conducted on five animals from the high-dose group. There was no change in water consumption, survival, behavior, body weight, or organ weights (liver, kidney, spleen, and testes) at any exposure. There was no evidence of hepatocellular necrosis at any exposure and only minimal changes in the levels of serum enzymes. This study identifies a NOAEL for noncancer effects in male mice of 146.6 mg/kg-day (the highest exposure tested). There was no increase in the prevalence of neoplasia at sites other than the liver. The male mice showed an increase of proliferative lesions in the liver (hyperplasia, adenoma, carcinoma, and combined adenoma and carcinoma) at all exposures. The prevalence of proliferative lesions in the control, 13.5, 65, or 146.6 mg/kg-day groups was as follows: hyperplasia, 3/42, 15/46, 13/39, 12/32; adenoma, 9/42, 20/46, 20/39, 16/32; carcinoma, 23/42, 25/46, 23/39, 27/32; adenoma or carcinoma, 27/42, 36/46, 31/39, 29/32. All of the changes were statistically significant ($p<0.05$) except for carcinoma at the two lower exposures.

NTP (2000a) conducted a chronic bioassay for carcinogenicity in female $B6C3F_1$ mice. Mice were administered chloral hydrate by gavage in distilled water at 0, 25, 50, or 100 mg/kg 5 days a week for up to 2 years. The calculated exposures are 0, 17.9, 35.7, or 71.4 mg/kg-day. Additional groups were administered chloral hydrate by gavage for 3, 6, or 12 months and held without further dosing for the duration of the study (stop-exposure studies). There was no significant effect on survival, body weight, or organ weights at any exposure. Following complete necropsy and histopathological examination, the only significant findings were in the pituitary gland pars distalis. There were no significant effects in the pituitary in the stop-exposure studies. Following the full exposure regime, the incidence of hyperplasia in the pituitary gland pars distalis was 4/45, 6/44, 4/50, and 9/50 in the control, 25, 50, and 100 mg/kg group, respectively. The average severity grade for hyperplasia was 1.5, 1.0, 1.0, and 2.2 in the control, 25, 50, and 100 mg/kg group, respectively. Only the average severity grade at 100 mg/kg was statistically different from the control ($p<0.05$). The incidence of adenoma in the pituitary gland pars distalis was 0/45, 2/44, 0/47, and 5/41, in the control, 25, 50, and 100 mg/kg group, respectively. Only at 100 mg/kg was the incidence significantly greater from the control ($p=0.0237$). For non-neoplastic effects, the NOAEL in this study is 71.4 mg/kg-day (the highest exposure tested). NTP concluded that this study provided equivocal evidence of carcinogenic activity for chloral hydrate in female mice.

NTP (2000b) conducted a chronic bioassay for carcinogenicity in male $B6C3F_1$ mice. Groups of 120 male mice received chloral hydrate by gavage in distilled water at 0, 25, 50, or 100 mg/kg for up to 2 years. The calculated exposures were 0, 17.9, 35.7, or 71.4 mg/kg-day. At

each exposure 60 mice received feed *ad libitum;* the other 60 mice received feed in a measured daily amount calculated to maintain body weight on a previously computed idealized body weight curve. Twelve mice from each diet and dose group were evaluated after 15 months of exposure. The remaining 48 animals from each diet and dose group were evaluated at 2 years. Survival, body weight, organ weights, and serum enzymes in the dosed groups were comparable to the respective vehicle control. Following complete necropsy and histopathological examination, no changes were found in any organ except the liver when compared to the respective vehicle control. The incidence of hepatocellular adenoma or carcinoma in the *ad libitum* study was 16/48, 25/48, 23/47, and 22/48 in the control, 25, 50, and 100 mg/kg groups, respectively. Only in the 25 mg/kg group was the incidence significantly greater from control (p=0.0437). In the dietary controlled study, the incidence of hepatocellular adenoma or carcinoma was 11/48, 11/48, 14/48, and 18/48, and the incidence of hepatocellular carcinoma was 2/48, 5/48, 4/48, and 8/48, in the control, 25, 50, and 100 mg/kg group, respectively. The only statistically significant increase in incidence was for hepatocellular carcinoma in the 100 mg/kg group (p = 0.042). The NOAEL for non-neoplastic effects in this study is 71.4 mg/kg-day (the highest exposure tested). NTP concluded that this study provided some evidence of carcinogenic activity for chloral hydrate in male mice.

Daniel et al. (1992b) exposed male and female Sprague-Dawley rats (10/sex/dose) for 90 days to chloral hydrate in drinking water at a concentration of 300, 600, 1,200, or 2,400 mg/L (equivalent to 24, 48, 96, or 168 mg/kg-day in males and 33, 72, 132, or 288 mg/kg-day in females). The tissues of animals from the high-exposure group and liver sections from all treated males were examined histopathologically. No mortality occurred in any groups prior to sacrifice. Organ weights, including liver weight, and clinical chemistry values in treated animals were only sporadically or inconsistently different from control animal values. Focal hepatocellular necrosis was observed in 2 of 10 males in each of the groups exposed to 96 and 168 mg/kg-day. The necrotic lesion was minimal at 96 mg/kg-day and was significantly more severe at 168 mg/kg-day. Necrotic lesions were not reported in any treated females or in any control animals. While serum enzymes were generally increased in treated animals, dramatic increases were reported in males in the 168 mg/kg-day group; mean aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase levels in this group were elevated 89%, 54%, and 127% above the corresponding control values, respectively. Based on the focal hepatocellular necrosis and accompanying serum enzyme changes, the study identifies a LOAEL of 168 mg/kg-day and a NOAEL of 96 mg/kg-day. The 96 mg/kg-day exposure is not considered a LOAEL because the authors reported only minimal microscopic necrosis, there was no corresponding increase in serum liver enzymes, and Sprague-Dawley rats showed no hepatocellular necrosis after chronic exposure to a higher daily exposure (Leuschner and Beuscher, 1998; George et al., 2000).

Leuschner and Beuscher (1998) conducted a chronic bioassay for carcinogenicity in Sprague-Dawley rats. Chloral hydrate was administered in drinking water for 124 weeks (males) and 128 weeks (females). The rats (50 males and 50 females in each group) received 15, 45, or 135 mg/kg-day. There was no effect on survival, appearance, behavior, body weight, food and water consumption, or organ weights. There was no increased incidence of tumors in any organ.

Histopathological examination revealed increased hepatocellular hypertrophy at the highest exposure in males only (11% in controls versus 28% at the highest exposure, $p<0.01$). This finding, graded as minimal to slight in severity, was characterized by a diffuse liver cell enlargement with slight eosinophilic cytoplasm and was considered by the authors as a first sign of toxicity. The type, incidence, and severity or other non-neoplastic lesions were not increased in treated animals compared to controls. Based on the evidence of minimal toxicity in the liver, which is of doubtful biological significance, the NOAEL in this study is 45 mg/kg-day; the LOAEL is 135 mg/kg-day.

George et al. (2000) conducted a chronic bioassay for carcinogenicity in male F344 rats. Rats were administered chloral hydrate in drinking water for 104 weeks. Rats (78 in each group) had a mean daily exposure of 0, 7.4, 37.4, or 162.6 mg/kg-day. At the termination of the study, a complete necropsy and histopathological examination of liver, kidney, spleen, and testes from all animals was conducted. In addition, a complete histopathological examination was conducted on five animals from the high-dose group. There was no change in water consumption, survival, behavior, body weight, or organ weights (liver, kidney, spleen, and testes) at any exposure. There was no indication of liver toxicity at any exposure, as shown by the lack of liver necrosis, hyperplasia, increased mitotic index, and only minimal changes in the levels of serum enzymes. There was no increase at any exposure in the prevalence or multiplicity of hepatocellular neoplasia or neoplasia at any other site. The NOAEL in this study is 162.6 mg/kg-day (the highest exposure tested).

Two of the metabolites of chloral hydrate, trichloroacetic acid and dichloroacetic acid, have been associated with increased hepatocellular adenomas or carcinomas in rodents. For example, trichloroacetic acid in drinking water induced hepatocellular adenomas or carcinomas in male and female mice when the exposure exceeded 200 mg/kg-day (Bull et al., 1990; Herren-Freund et al., 1987; Pereira, 1996). There was no evidence of increased carcinogenicity, however, when male rats were exposed to trichloracetic acid at 360 mg/kg-day (DeAngelo et al., 1997). Dichloroacetic acid in drinking water induced hepatocellular adenomas or carcinomas in male and female mice when the exposure exceeded 160 mg/kg-day (Bull et al., 1990; Daniel et al., 1992a; DeAngelo et al., 1991; Ferreira-Gonzalez et al., 1995; Herren-Freund et al., 1987; Pereira, 1996). Dichloroacetic acid also induced hepatocellular adenomas or carcinomas in male rats when the exposure exceeded 40 mg/kg-day (DeAngelo et al., 1996; Richmond et al., 1995).

4.2.2. Inhalation

No studies on chloral hydrate were located. One study on chloral was available. Odum et al. (1992) exposed four female CD-1 mice to chloral for 6 hours at a concentration of 100 ppm (603 mg/m^3). This exposure induced deep anesthesia. The mice recovered normally after the exposure stopped. The effects in the lung included vacuolization of clara cells, alveolar necrosis, desquamination of the epithelium, and alveolar edema. The lung-to-body weight ratio increased 1.5-fold, most likely because of the alveolar edema.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

Klinefelter et al. (1995) evaluated sperm morphology and motility in F344 rats administered chloral hydrate in drinking water for 52 weeks at 0, 55, or 188 mg/kg-day. The researchers examined cauda epididymal sperm motion parameters and testicular and epididymal histopathology. Chloral hydrate did not cause any visible systemic toxicity and had no effects on epididymal or testicular histopathology. However, the percentage of motile sperm was significantly decreased ($p<0.01$) from 68% in controls to 58% in rats exposed to 188 mg/kg-day. The percentage of progressively motile sperm was also significantly decreased ($p<0.01$) from 63% in controls to 53% in this group. In addition, the frequency distribution of the average straight-line velocities of sperm at this exposure was significantly shifted ($p<0.01$) to the lower ranges when compared to controls. In this study the NOAEL is 55 mg/kg-day; the LOAEL is 188 mg/kg-day.

Kallman et al. (1984) exposed male and female CD-1 mice to chloral hydrate in drinking water at 21.3 or 204.8 mg/kg-day. Animals were exposed for 3 weeks prior to breeding. Exposure of females (5 per group) continued during gestation and until pups were weaned at 21 days of age. There was no change in drinking water consumption or weight gain in the dams. No gross malformations were noted in pups, and no significant effects were observed in duration of gestation, number of pups delivered, pup weight, or number of stillborn pups. All pups (15 per group) showed the same rate of development and level of performance on several neurobehavioral tests, except that pups exposed to 204.8 mg/kg-day when tested at 23 days of age showed impaired retention of passive avoidance learning on both the 1-hour and 24-hour retention tests ($p<0.05$). This study identified a NOAEL for neurodevelopmental toxicity of 21.3 mg/kg-day and a LOAEL of 204.8 mg/kg-day based on the impairment in passive avoidance learning. This study also identifies a NOAEL for reproductive and other developmental effects of 204.8 mg/kg-day (the highest exposure tested).

Johnson et al. (1998) tested the potential for chloral hydrate to cause developmental toxicity in Sprague-Dawley rats. Chloral hydrate was administered in drinking water to 20 rats from gestational day 1 to 22 at an average exposure of 151 mg/kg-day. Control animals were given distilled water. There was no evidence of maternal toxicity, no change in the number of implantation or resorption sites, no change in the number of live or dead fetuses, no change in placental or fetal weight, no change in crown-rump length, and no increase in the incidence of morphological changes. At necropsy there was no evidence of cardiac anomalies. Based on this study, the NOAEL for developmental toxicity is 151 mg/kg-day (the highest exposure tested).

Johnson et al. (1998) also tested the potential for trichloroethanol and trichloroacetic acid to cause developmental toxicity in Sprague-Dawley rats. The protocol was identical to the study with chloral hydrate. Trichloroethanol was administered to 10 rats at an average exposure of 153 mg/kg-day. No evidence of developmental toxicity was found. In contrast, when trichloroacetic acid was administered to 11 rats at an average exposure of 291 mg/kg-day, developmental toxicity was observed. The effects included a statistically significant ($p<0.05$) increase in total

cardiac defects per litter and an increased number of implantation and resorption sites. The results with trichloroacetic acid are generally consistent with those reported by Smith et al. (1989), who reported adverse developmental effects (levocardia) from trichloroacetic acid at an exposure of 330 mg/kg-day and above.

Saillenfait et al. (1995) tested the potential of chloral hydrate to cause developmental toxicity in vitro using a rat whole embryo culture system. Embryos (20/dose) from Sprague-Dawley rats were explanted on gestational day 10 and exposed to chloral hydrate at a concentration of 0, 0.5, 1, 1.5, 2, or 2.5 mM (equivalent to 83, 165, 248, 331, or 414 mg/L) for 46 hours. At 2.5 mM all embryos died. No lethality was seen at lower exposures. Chloral hydrate caused concentration-dependent decreases in growth and differentiation and increases in the incidence of morphologically abnormal embryos. No effects were observed in any parameter at 0.5 mM. Decreases in crown-rump length, somite (embryonic segment) number, and the protein or DNA content of embryos were seen at 1 mM and above. At 1, 1.5, and 2 mM chloral hydrate, respectively, 18%, 68%, and 100% of embryos were malformed. Brain, eye, and ear malformations were the most prominent effects at these concentrations. Abnormalities in the trunk and pericardial dilation also occurred at 2 mM. In this in vitro test system, chloral hydrate was a slightly more potent teratogen than trichloroacetic acid or dichloroacetic acid.

Although chloral hydrate did not cause meiotic delay in the oocytes of adult mice when administered at the time of resumption of maturation induced by hormones (Mailhes et al., 1994), it did cause adverse effects in vitro when a synchronized population of oocytes was exposed prior to resumption of maturation (Eichenlaub-Ritter and Betzendahl, 1995; Eichenlaub-Ritter et al., 1996). In this test system, chloral hydrate induced lagging of chromosomes during telophase I, inhibited spindle elongation in anaphase B, and caused chromosome displacement from the spindle equator in metaphase I and II. Oocytes became irreversibly arrested in maturation when exposed to chloral hydrate prior to resumption of maturation, or when chloral hydrate was present during the first or second 8 hours of maturation. Spindle aberrations (lagging chromosomes and a short interpolar space) were observed when oocytes were treated with trichloroethanol (Eichenlaub-Ritter et al., 1996).

4.4. OTHER STUDIES—NEUROLOGICAL, IMMUNOLOGICAL, GENOTOXICITY, MECHANISTIC

4.4.1. Neurological Studies

Kallman et al. (1984) administered chloral hydrate by gavage in distilled water at 50, 100, 200, 300, or 400 mg/kg to groups of 12 seven-week-old male CD-1 mice. All doses resulted in the rapid onset of ataxia, with an ED_{50} of 84.2 mg/kg at 5 minutes (the time of maximal effect). Animals recovered within 2 to 3 hours. No delayed changes in muscular coordination were detectable when the mice were tested 24 hours after treatment.

Kallman et al. (1984) evaluated the potential behavioral toxicity in groups of 12 seven-week-old male CD-1 mice administered chloral hydrate by gavage in distilled water at 14.4 or 144 mg/kg-day for 14 days. When measurements were made 24-48 hours after the 14 day exposure was terminated, no significant effects were observed on body weight, motor activity, physical appearance, behavior, muscular coordination, or endurance.

Kallman et al. (1984) exposed groups of 12 five-week-old male CD-1 mice to drinking water containing chloral hydrate at a concentration of 70 or 700 mg/L (equivalent to 15.7 or 160 mg/kg-day) for 90 days. When measurements were made 24 hours after the 90-day exposure was terminated, no treatment-related effects were observed on mortality, body weight, physical appearance, behavior, locomotor activity, learning in repetitive tests of coordination, response to painful stimuli, strength, endurance, or passive avoidance learning. Both exposures resulted in a decrease of about 1°C in mean body temperature ($p<0.05$). Because of the lack of increased effect with a tenfold increase in exposure and because hypothermia as a side effect of chloral hydrate or from an overdose of chloral hydrate has not been reported in humans, the decrease in body temperature is not considered an adverse effect. This study identifies a NOAEL for neurobehavioral toxicity of 160 mg/kg-day (the highest exposure tested).

4.4.2. Immunological Studies

Kauffmann et al. (1982) administered chloral hydrate by gavage in distilled water at 14.4 or 144 mg/kg-day to groups of 11 to 12 six-week-old male CD-1 mice for 14 days. No effects on humoral or cell-mediated immunity were detected at either exposure.

Kauffmann et al. (1982) administered chloral hydrate to male and female 4-week-old CD-1 mice in drinking water at 70 or 700 mg/L (equivalent to 16 or 160 mg/kg-day) for 90 days. Humoral immunity was assessed by the number of splenic antibody-forming cells produced against sheep red blood cells (12 mice in the control group and 8 mice in the exposed groups) and hemagglutination titers (20-21 mice in the control group and 13-16 mice in the exposed groups). Cell-mediated immunity was assessed by delayed type hypersensitivity to sheep red blood cells (17-20 mice in the control group and 15-16 mice in the exposed groups). Lymphocyte response was assessed using a T-cell mitogen (Con A) and a B-cell mitogen (LPS) (17-22 animals in the control group and 13-16 mice in the exposed groups). In males, no effects were detected in either humoral or cell-mediated immunity at either exposure. No effects on cell-mediated immunity were noted in females at either exposure. In females, both exposures resulted in a statistically significant decrease ($p<0.05$) in humoral immune function (36% and 40% at the low and high exposure, respectively) when expressed as antibody-forming cells per spleen. The decrease, however, was statistically significant only at the higher exposure when expressed as antibody forming cells per million spleen cells (a 32% decrease). There was no effect on hemagglutination titers or on spleen cell response to the B-cell mitogen at either exposure. The decrease in antibody-forming cells per million spleen cells at the higher exposure in female mice is regarded as an adverse response in this study. Accordingly, the NOAEL for immunotoxicity is 16 mg/kg-day; the LOAEL is 160 mg/kg-day.

4.4.3. Genetic Toxicity

There is an extensive database on the genotoxicity of chloral hydrate and its metabolites. A summary is provided in Table 1. The European Union included chloral hydrate in a collaborative study on aneuploidy (Adler, 1993; Natarajan et al., 1993; Parry, 1993; Parry and Sors, 1993).

Chloral hydrate did not induce mutation in most strains of *Salmonella typhimurium*, but did in some studies with *Salmonella typhimurium* TA 100 and in a single study with *Salmonella typhimurium* TA 104. The latter response was inhibited by free-radical scavengers "-tocopherol and menadione (Ni et al., 1994).

Chloral hydrate did not induce mitotic crossing over in *Aspergillus nidulans* in the absence of metabolic activation. Chloral hydrate caused weak induction of meiotic recombination in the presence of metabolic activation and gene conversion in the absence of metabolic activation in *Saccharomyces cerevisiae*. It did not induce reverse mutation in *Saccharomyces cerevisiae*. Chloral hydrate clearly induced aneuploidy in various fungi in the absence of metabolic activation.

Chloral hydrate induced somatic and germ cell mutations in *Drosophila melanogaster*.

Choral hydrate did not produce DNA-protein cross-links in rat liver nuclei, DNA single-strand breaks/alkaline-labile sites in primary hepatocytes in vitro, or DNA repair in *Escherichia coli*. One study showed induction of single-strand breaks in liver DNA of both rats and mice treated in vivo; another study in both species using higher concentrations of chloral hydrate found no such effect.

Chloral hydrate was weakly mutagenic, but did not induce micronuclei in mouse lymphoma cells in vitro. Chloral hydrate increased the frequency of micronuclei in Chinese hamster cell lines. Although a single study suggested that chloral hydrate induces chromosomal aberrations in Chinese hamster CHED cells in vitro, the micronuclei produced probably contained whole chromosomes and not chromosome fragments, as the micronuclei could all be labeled with antikinetochore antibodies.

In kangaroo rat kidney epithelial cells, choral hydrate inhibited spindle elongation and broke down mitotic microtubuli, although it did not inhibit pole-to-pole movement of chromosomes. It produced multipolar spindles, chromosomal dislocation from the mitotic spindle, and a total lack of mitotic spindles in Chinese hamster DON:Wg3h cells.

Chloral hydrate weakly induced sister chromatid exchange in cultured human lymphocytes. It induced micronuclei, aneuploidy, C-mitosis, and polyploidy in human lymphocytes in vitro. Micronuclei were induced in studies with human whole-blood cultures, but not in one study with isolated lymphocytes. The differences seen in the micronucleus test have

been attributed to differences between whole-blood and purified lymphocyte cultures (Vian et al., 1995), but this hypothesis has not been tested.

Chloral hydrate increased the frequency of chromosomal aberrations in mouse bone marrow, spermatogonia, and primary and secondary spermatocytes, but not in oocytes, after in vivo treatment. Chloral hydrate induced chromosomal aberrations in mouse bone-marrow erythrocytes after treatment in vivo. Chloral hydrate induced micronuclei in the spermatids of mice treated in vivo in some studies. Chloral hydrate induced aneuploidy in the bone marrow of mice treated in vivo. Chloral hydrate increased the rate of aneuploidy in mouse secondary spermatocytes. Chloral hydrate did not produce polyploidy in bone marrow, oocytes, or gonosomal or autosomal univalents in primary spermatoctyes of mice treated in vivo. Chloral hydrate, however, induced polyploidy and meiotic delay when a synchronized population of mouse oocytes were exposed in vitro prior to the resumption of maturation.

Trichloroethanol, a reduction product of chloral hydrate, did not induce 8 prophage in *Escherichia coli* or mutation in *Salmonella typhimurium* TA 100. Trichloroethanol caused spindle aberrations (lagging of chromosomes and a short interpolar space) when mouse oocytes were treated in vitro.

Trichloroacetic acid did not induce 8 prophage in *Escherichia coli* and was not mutagenic to *Salmonella typhimurium* in the presence or absence of metabolic activation. Trichloroacetic acid was weakly positive in the mouse lymphoma assay with metabolic activation. Trichloroacetic acid also did not induce chromosomal damage in human lymphocytes or micronuclei in bone marrow in vitro. It is unclear whether trichloroacetic acid can induce chromosomal damage in vivo because some studies have been positive and other negative.

Dichloroacetic acid did not induce differential toxicity in DNA-repair deficient strains of *Salmonella typhimurium* but did induce 8 prophage in *Escherichia coli*. Dichloroacetic acid gave equivocal results for gene mutation in *Salmonella typhimurium* TA100 and TA98. Dichloroacetic acid was weakly mutagenic in the in vitro mouse lymphoma assay and induced chromosomal aberrations but not micronuclei or aneuploidy in that test system. Dichloroacetic acid induced micronuclei in mouse polychromatic erythrocytes in vivo and mutations at the LacI locus in the transgenic B6C3F$_1$ mouse (Big Blue® mouse) in vivo at an exposure that induces liver tumors in male mice. It is unclear whether dichloroacetic acid can induce primary DNA damage, as some assays are positive and others negative.

4.4.4. Mechanistic Studies

4.4.4.1. *Cell Proliferation*

Rijhsinghani et al. (1986) evaluated the acute effects of chloral hydrate on liver cell proliferation in 15-day-old male C57BL × C3HF$_1$ mice. Mice were given a single dose of 0, 5, or

10 mg/kg chloral hydrate by gavage in distilled water (9, 10, and 6 mice per group, respectively) and sacrificed after 24 hours. Cell proliferation was evaluated by calculating the mitotic index (number of mitoses/100 nuclei) from liver sections. The mitotic index in liver cells was significantly increased (0.9235) in mice receiving 5 mg/kg when compared to the control value (0.3382), and elevated (0.7433) (although not statistically significantly) in mice receiving 10 mg/kg. Hepatic necrosis was not observed in mice from either treatment group at autopsy.

As part of the chronic bioassay for carcinogenicity, George et al. (2000) evaluated hepatocyte proliferation in F344 rats and B6C3F_1 mice. Exposures are given in Section 8.4.2. Five days prior to sacrifice at 13, 26, 52, or 72 weeks in rats and 26, 52, or 78 weeks in mice, animals were given bromodeoxyuridine. Labeled nuclei were identified by chromogen pigment over the nuclei and the labeling index was calculated. Outside of the areas with tumors in the livers of male mice, there was no significant evidence of increased hepatocyte proliferation in rats or mice.

4.4.4.2. *Oncogene Activation*

Velazquez (1994) investigated the induction of H-*ras* proto-oncogene mutations in mice. DNA from normal liver and tumor tissue was obtained from male B6C3F_1 mice administered 1 g/L (166 mg/kg-day) chloral hydrate in drinking water for 2 years. H-*ras* mutations were present in one out of seven (14%) tumors. The spectrum of mutations was the same as that of spontaneous liver tumors. Based on these data, it is unlikely that H-*ras* activation is a mechanism of potential carcinogenicity relevant to chloral hydrate.

4.4.4.3. *Free Radicals and DNA Adduct Formation*

Ni et al. (1994, 1995, 1996) studied the metabolism of chloral hydrate in an in vitro system using microsomes from male B6C3F_1 mice. The metabolism of chloral hydrate generated free radicals as detected by electron spin resonance spectroscopy and caused endogenous lipid peroxidation, resulting in the production of malondialdehyde, formaldehyde, and acetaldehyde, all of which are known to produce liver tumors in rodents. Trichloroacetic acid and trichloroethanol also produced free radicals and induced lipid peroxidation when tested in this system. The authors speculated that the free radicals were Cl_3CCO_2• and/or Cl_3C•. Incubation of chloral hydrate, trichloroethanol, or trichloroacetic acid in the presence of microsomes and calf thymus DNA resulted in the formation of a malondialdehyde-modified DNA adduct. This research group further showed that chloral hydrate induced an increase in mutations at the *hprt* and *tk* loci in transgenic human lymphoblastoid cells containing CYP2E1. In contrast, when the parental cell line lacking CYP2E1 was treated with the same concentration of chloral hydrate, no mutations were found at either locus. These data implicate CYP2E1 as the primary cytochrome subfamily involved in the metabolism of chloral hydrate to reactive intermediates.

4.4.4.4. *Cell Communication*

The effects of 1-, 4-, 6-, 24-, 48-, and 168-hour exposures to chloral hydrate (0, 1, 5, or 10 mM) on gap junction intercellular communication in Clone 9 cell cultures (normal rat hepatocytes) were reported by Benane et al. (1996). No differences in intercellular communication were seen between the groups treated with 1 mM at 1, 4, and 6 hours of exposure and controls, as measured by a dye transfer protocol. There were significant differences between all other groups and the controls. The shortest exposure time and lowest exposure concentration that reduced dye transfer significantly was in the group treated with 1 mM for 24 hours.

4.4.4.5. *Peroxisome Proliferation*

As part of the chronic bioassay for carcinogenicity in mice, George et al. (2000) found no evidence of peroxisome proliferation using cyanide-insensitive palmitoyl CoA oxidase in the livers of male mice treated with chloral hydrate for 26 weeks. As part of the chronic bioassay for carcinogenicity in male mice, NTP (2000b) found that chloral hydrate in the 100 mg/kg group significantly ($p<0.05$) induced both lauric acid T-hydroxylase activity and CYP4A immunoreactive protein in the dietary-controlled study, but not in the *ad libitum* study.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)

Chloral hydrate has been used for a great many years as a sedative/hypnotic drug in human and veterinary medicine. The metabolite, trichloroethanol, is responsible for the pharmacological effect. The proposed mechanisms for the depression of the central nervous system include potentiating the function of $GABA_A$ receptors (Lovinger et al., 1993), inhibition of excitatory amino acid-activated currents mediated by N-methyl-D-aspartate (Peoples and Wright, 1998; Scheibler et al., 1999), and allosteric modulation of the 5-hydroxytryptamine$_3$ receptor-mediated depolarization of the vegas nerve (Bentley and Barnes, 1998).

Chloral hydrate is corrosive and irritating to the skin and mucous membranes and can cause gastric distress, nausea, and vomiting at recommended doses. Acute overdoses produce (in order of progression) ataxia, lethargy, deep coma, respiratory depression, hypotension, and cardiac arrhythmias. There is some evidence of hepatic injury in people surviving near lethal, acute overdoses, but no convincing evidence that hepatic injury results at the recommended clinical dose. Despite its long use in human medicine, there is no published information on toxicity in controlled studies in humans following extended exposure.

Chloral hydrate is completely absorbed and rapidly metabolized following oral administration. The major metabolites are trichloroethanol and its glucuronide and trichloroacetic acid. Some data suggest a small amount of dichloroacetic acid may be formed. In humans the half-life of trichloroethanol and its glucuronide is about 8 hours; the half-life of

trichloroacetic acid is about 4 days. Some data suggest that the half-life of trichloroethanol is increased several-fold in preterm and full-term infants compared to toddlers and adults. The major route of excretion of the metabolites of chloral hydrate is the urine. Chloral hydrate and its metabolites have been found in milk from women treated with chloral hydrate. The concentration of these chemicals, however, is too low to cause a pharmacological effect in the nursing infant.

Acute administration of chloral hydrate to mice causes loss of coordination (ataxia) at about the same exposure as in humans for the same effect. A 90-day study in mice shows no evidence of behavioral changes or other neurotoxicity. Chronic studies in rats and mice show no evidence of behavioral changes and no evidence of histopathological changes in nervous tissue. There is some evidence of mild liver toxicity following chronic exposure in rats and mice. A slight decrement in humoral immunity was observed in female mice following exposure for 90 days. The antibody-forming cell response is considered an excellent indicator of the status of humoral immunity because of the complex cellular cooperation required to produce antibody and because the number of cells that produce antibody can be quantified. A depression in the number of these cells is considered an adverse response because the production of antibodies is important to the defense strategy of the organism. However, the quantitative relationship between the depression in antibody-forming cells in the spleen and the concentration of circulating antibody is unknown. In this study, because there was no depression in circulating antibodies measured by the hemagglutination titer, there might be no significant depression in the ability of the host to mount a protective antibody response. Chloral hydrate has been tested for developmental effects in rats and mice. No structural abnormalities were observed. A slight effect was observed in mice in passive avoidance learning when dams were exposed prior to breeding, during gestation and nursing, and pups were tested at 23 days of age. Although chloral hydrate has not been tested in a two-generation reproduction study, the data on reproductive performance and on effects on sperm and oocytes do not suggest that reproductive toxicity is likely to be a critical effect. In addition, no histopathological effects are observed in reproductive organs of rodents in subchronic or chronic studies. All of the studies in laboratory animals show noncancer health effects at an exposure far in excess of the exposure that causes CNS depression and gastrointestinal irritation in humans. See Table 2.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION—SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

There are no carcinogenicity data from humans. Two bioassays in rats show no increase in tumors at any site. Because only minimal toxicity was observed in the livers of the rats in these bioassays, the tests were not conducted at the maximum tolerated dose. A chronic bioassay in female mice showed a slight increase in the severity of hyperplasia and a slight increase in the incidence of adenoma in the pituitary gland pars distalis at the highest exposure tested. There is

some evidence that chloral hydrate causes hepatocellular tumors in male mice. An earlier study showing an increase in hepatic adenomas or trabecular carcinomas following a single bolus exposure could not be confirmed in a study using more animals and higher exposures. Three separate 2-year bioassays in male mice show an increased incidence of hepatocellular adenoma or carcinoma. There are no data identifying a lesion that is a precursor to the hepatocellular tumors. The strain of mice used has a very high spontaneous incidence of hepatocellular tumors. Two of the metabolites of chloral hydrate, trichloroacetic acid and dichloroacetic acid, have been shown to cause hepatocellular tumors in rodents. Trichloroacetic acid causes hepatocellular tumors only in mice. Dichloroacetic acid causes hepatocellular tumors in both rats and mice.

Chloral hydrate has been extensively studied as a genotoxic agent. Chloral hydrate was positive in some bacterial mutation tests, indicating that it may be capable of inducing point mutations. It was also positive in the mouse lymphoma assay for mutations at the TK locus. Chloral hydrate induced somatic and germ cell mutations in *Drosophila melanogaster*. Some data also show chloral hydrate to be a very weak clastogen in mammalian cells.

Chloral hydrate has been shown to induce aneuploidy in a variety of cells, including *Saccharomyces cerevisiae*, *Aspergillus nidulans*, Chinese hamster embryonic fibroblasts, Chinese hamster primary cell lines LUC2 and DON:Wg3h, human peripheral blood lymphocytes, mouse spermatocytes, and mouse spermatids. Because there is a mixture of positive and negative in vivo data, with no reason to weigh some studies more than others, it is not clear whether chloral hydrate is capable of inducing genetic damage in vivo. Additional in vivo studies using standard protocols would help clarify the relevance of genetic damage to a human health risk assessment.

The aneugenic effects of chloral hydrate are exposure-dependent and thought to arise via disruption of the mitotic spindle structure and/or function by inhibition of tubulin and/or microtubule-associated proteins; both substances are components of the spindle apparatus (Brunner et al., 1991; Lee at al., 1987; Wallin and Hartley-Asp, 1993). Some data also suggest that chloral hydrate may act on the spindle apparatus through an increase in the concentration of intracellular free calcium (Lee et al., 1987).

Although chloral hydrate and its metabolites, trichloroacetic acid and dichloroacetic acid, can induce a variety of mutational events, they do so with very low potency. Owing to the high concentration of chloral hydrate and its metabolites required to induce an observable effect in these assays, it is not likely that a genotoxic mode of action can be held responsible for the pituitary adenomas found in female mice or the hepatocellular tumors found in male mice.

Several other mechanisms may play a role in the induction of tumors in the liver of male mice. There is no convincing evidence that chloral hydrate causes direct damage to DNA. In vitro studies with chloral hydrate, trichloroethanol, and trichloroacetic acid and mouse microsomes, however, show lipid peroxidation and formation of covalently bound DNA adducts. These effects appear to be mediated by the formation of free radicals by CYP2E1. Another

possibility concerns exposure-dependent cytotoxicity leading to compensatory hyperplasia. A single treatment of mice with chloral hydrate caused an increase in the mitotic index in liver cells. The increased cell division is hypothesized to either provide additional opportunities for errors in DNA replication or allow initiated cells to progress to a tumor. Some data suggest a role for peroxisomal proliferation in the liver of male mice. Another potentially contributing mechanism of carcinogenesis is disruption of intercellular communication, which has been shown in one experiment to be influenced by chloral hydrate.

Although the mechanism of chloral hydrate-induced carcinogenicity in mice is unclear, one mechanism that appears less likely to be responsible is H-ras proto-oncogene activation.

Collectively, these data provide suggestive evidence of carcinogenicity in mice, but the weight of evidence is not sufficient to conclude that carcinogenesis is the critical effect.

4.6.1. Susceptible Populations

Simultaneous ingestion of ethanol and chloral hydrate increases the sedative and side effects of chloral hydrate. The mechanism is the increase in the concentration of the pharmacologically active metabolite, trichloroethanol, in the presence of ethanol. Chronic abusers of ethanol are, therefore, somewhat more sensitive to the adverse effects of chloral hydrate.

EPA is not aware of any studies showing increased susceptibility to chloral hydrate in individuals with genetic polymorphisms in the enzyme that metabolize chloral hydrate. EPA believes that individuals with deficiencies in alcohol dehydrogenase or aldehyde dehydrogenase will not be at increased risk from the effects of chloral hydrate when exposure is at or below the reference dose. Individuals with deficiencies in alcohol dehydrogenase will be less able to metabolize chloral hydrate to trichloroethanol. However, because chloral hydrate is rapidly excreted by the kidney, it is unlikely that these individuals will experience greater or more prolonged central nervous system depression or potential adverse effects from the increase in the amount of trichloroacetic acid formed. Similarly, individuals with deficiencies in aldehyde dehydrogenase will be less able to metabolize chloral hydrate to trichloroacetic acid. However, as 92% of the chloral hydrate is converted to trichloroethanol, the slight increase in the trichloroethanol concentration in these individuals has no biological significance.

4.6.1.1. *Possible Childhood Susceptibility*

Because of the immaturity of hepatic metabolism, particularly the glucuronidation pathway, and decreased glomerular filtration, the half-life of trichloroethanol is longer in infants (preterm and full-term) than in adults. The half-life of trichloroethanol in toddlers and adults is similar. Because of the longer half-life of trichloroethanol, pre-term and full term infants will experience prolonged effects when chloral hydrate is administered. However, at the reference

dose for chloral hydrate, the steady-state concentration of trichloroethanol in these groups is far below the concentration required for the pharmacological effect. See Appendix B.

4.6.1.2. *Possible Gender Differences*

Although male laboratory rodents seem to be more sensitive than female laboratory rodents to hepatic effects, there is no evidence of a gender effect in humans to the sedative or side effects of chloral hydrate at the recommended clinical dose.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

The effect that occurs at the lowest exposure is CNS depression and gastrointestinal irritation in humans. As these effects would not be intended or desirable in the general population, EPA considers these responses as adverse effects and uses them to derive the reference dose.

Acute gavage exposure in mice shows neurological effects (ataxia) at about the same exposure for the comparable effect in humans. A subchronic study in mice using sensitive tests for neurobehavioral changes found none. Chronic studies in rats and mice show no evidence of neurobehavioral changes and no evidence of histopathological changes in nervous tissue. As with other chlorinated chemicals, there is some evidence of hepatotoxicity in rodent liver following chronic oral exposure. These effects are of minimal severity, may be related to precancerous lesions, and occur at an exposure greater than that required for CNS depression and gastrointestinal irritation following an acute bolus dose.

5.1.2. Methods of Analysis—Including Models (PBPK, BMD, etc.)

No data are available to determine a NOAEL in humans. The recommended clinical dose for sedation in adults is 250 mg, taken 3 times a day (Goodman and Gilman, 1985). The LOAEL is 10.7 mg/kg-day (assuming a 70 kg body weight). The pharmacokinetic information shows that chloral hydrate and the pharmacologically active metabolite, trichloroethanol, will not bioaccumulate. See Appendix B.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UF) and Modifying Factors (MF)

The reference dose of 0.1 mg/kg-day was estimated from the LOAEL of 10.7 mg/kg-day using a total uncertainty factor (UF) of 100 and a modifying factor (MF) of 1. An uncertainty

factor of 10 was used to extrapolate from a LOAEL to NOAEL. An uncertainty factor of 10 was used for intraspecies variability. An uncertainty factor for chronic duration is not used. Chloral hydrate and the active metabolite, trichloroethanol, do not bioaccumulate. Therefore, continuous daily exposure to chloral hydrate at the reference dose will not result in a concentration of trichloroethanol in the blood required for the pharmacological effect. Developmental toxicity, including developmental neurotoxicity, and immunotoxicity are not critical effects. Although there is no two-generation reproduction study, a UF for database limitations is not needed, as there is evidence from several studies that reproductive toxicity is not likely to be a critical effect.

Although the reference value of 0.1 mg/kg-day derived from the pharmacologically active dose in humans is an acute RfD, keeping exposure below this level will also be protective for any noncancer health effect from chronic exposure. For example, chronic exposure to chloral hydrate does not cause adverse effects in the liver of rats or mice until the exposure approaches 135 or 160 mg/kg-day, respectively. Similarly, there are no reproductive, developmental, neurobehavioral, or immunological effects following long-term treatment of laboratory animals until the exposure approaches 160 mg/kg-day. See Table 2. Therefore, it is appropriate to use the acute RfD also as the chronic RfD.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

There are no inhalation studies adequate for establishing a reference concentration.

5.3. CANCER ASSESSMENT

No adequate data are available to calculate an oral slope factor or an inhalation unit risk.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Chloral hydrate has a long history of use in human and veterinary medicine as a sedative/hypnotic drug. Its metabolite, trichloroethanol, is responsible for the pharmacological action in the central nervous system. Chloral hydrate is completely absorbed following oral exposure and is rapidly distributed to all major tissues. Chloral hydrate is rapidly metabolized in the blood and liver. The major route of excretion of metabolites is the urine. Chloral hydrate and its metabolites have been found in milk from women treated with chloral hydrate, but below the concentration that would cause sedation in the nursing infant. Chloral hydrate and trichloroethanol do not bioaccumulate.

There are no controlled studies on toxicity to humans following extended exposure to chloral hydrate. Studies in laboratory animals demonstrate that liver is a target tissue and hepatocellular tumors have been observed in male mice and adenomas in the pituitary gland pars distalis in female mice after chronic, high dose administration. No tumors occurred in rats after chronic high-dose administration. Slight effects are also observed in some studies in laboratory animals on sperm motility, developmental neurotoxicity (passive avoidance learning), and humoral immunity. All of the adverse effects noted in studies in laboratory animals occur at an exposure that is greater than the recommended clinical dose for sedation in humans.

6.2. DOSE RESPONSE

The quantitative estimate of human risk for noncancer effects is based on the recommended clinical dose for sedation in humans. At this exposure the adverse effects are central nervous system depression and gastrointestinal irritation. The reference dose is 0.1 mg/kg-day. This is 1/100 of the recommended daily dose for sedation in humans.

Although there is suggestive evidence of formation of tumors in mice and some data showing aneugenicity, the mode of action for the formation of tumors is not known. It is also not known whether this response is relevant for humans. On the basis of available data, the most likely mode of action for the formation of tumors in mice involves interaction with cellular enzymes and proteins in contrast to direct interaction with DNA. These effects are expected to show a nonlinear response at low exposure.

Millions of people are exposed to chloral hydrate on a daily basis because it is formed during the disinfection of drinking water with chlorine. The typical concentration in a public water supply in the United States is 5 µg/L (US EPA, 1994e). Assuming a water consumption of 2 L/day and a body weight of 70 kg, the exposure is 0.00014 mg/kg-day. This exposure is approximately 700 times lower than the reference dose.

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APPENDIX A. EXTERNAL PEER REVIEW— SUMMARY OF COMMENTS AND DISPOSITION

The reviewers made a number of editorial suggestions and other suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

The reviewers were satisfied that the relevant literature was included. One reviewer indicated that NCTR had conducted chronic bioassays on chloral hydrate and requested that EPA incorporate information from these studies if final reports became available in a timely manner.

EPA has incorporated the results of these studies and NTP's conclusions, based on the peer review conducted on May 18, 2000, in this report.

Although the reviewers were satisfied that the most appropriate critical effect was used to derive the reference dose, one reviewer requested that more emphases be placed on gastrointestinal irritation (nausea, vomiting, diarrhea). These effects are directly caused by chloral hydrate and occur before absorption and metabolism to trichloroethanol. Another reviewer pointed out that the critical effect is central nervous system depression. The reviewer further pointed out that although sedation and CNS depression are related, the quantitative relationship between the effects is not known for chloral hydrate.

EPA agrees with the reviewers and has revised the document to list CNS depression and gastrointestinal irritation as the critical effects.

Two reviewers questioned why a dated version of Goodman and Gilman was cited as the source for the LOAEL for chloral hydrate. They suggested consulting an FDA summary on chloral hydrate, consulting the Physicians Desk Reference, or conducting a critical evaluation of the human clinical literature.

EPA found no useful information in current versions of the Physicians Desk Reference. EPA found that the information in Goodman and Gilman corresponded to information found in the clinical publications cited in the review. EPA therefore did not feel it necessary to evaluate the publications over the more than 130 years of clinical use of chloral hydrate. EPA was advised by FDA that because chloral hydrate is a pre-1938 drug product, a summary for chloral hydrate is not available. FDA advised that old versions of Goodman and Gilman could be considered to contain authoritative information on chloral hydrate.

Two reviewers asked for more effective use of the toxicokinetic calculations in Appendix B and the Benchmark Dose calculations in Appendix C.

EPA has incorporated more information from Appendix B, but has decided to delete Appendix C from the document. Appendix C contained the calculations for the exposure-

response relation for hepatocellular tumors from George et al. (2000). That information was included in the peer review draft in case reviewers had questions about the exposure-response relation and thought that chloral hydrate should be assigned to Cancer Group B2 (probable human carcinogen). As the reviewers agreed that chloral hydrate was properly assigned to Cancer Group C (possible human carcinogen) under the 1986 Cancer Guidelines [suggestive evidence of carcinogenicity under the 1996 proposed Cancer Guidelines], information on the exposure-response relation for tumors is no longer necessary, as it is not appropriate to derive a cancer slope factor or unit risk for a chemical showing only suggestive evidence of carcinogenicity.

Two reviewers wanted more scientific justification for the specific values of 10 used for the uncertainty factors for the LOAEL to NOAEL extrapolation and for intraspecies variability. One of these reviewers also requested a discussion of the known range of genetic polymorphisms in different races for the enzymes involved in the metabolism of chloral hydrate, as well as a discussion of whether individuals with hepatic diseases or infants with respiratory insufficiency or compromised hepatic and/or renal capabilities are adequately accounted for in the tenfold factor used for intraspecies variability.

EPA used values of 10 for these two uncertainty factors because there was insufficient reason to depart from the defaults. A greater justification would have been provided if some lesser value (3 or 1) had been used. EPA is not aware of any studies with chloral hydrate in different races that would provide definitive information to answer the reviewer's question. EPA, however, expanded Section 4.6.1 to discuss the issues raised by the reviewer. EPA has revised Section 4.6.1.1 to consider the increased half-life for elimination of trichloroethanol in preterm and full-term infants. The toxicokinetic analysis shows that the increased concentration of trichloroethanol in these subgroups is below the concentration required for a pharmacological effect. Finally, it has not been EPA's practice to ensure protection of 100% of the population from exposure to a chemical from the environment.

One reviewer wanted more discussion, justification, and explanation for using the acute RfD as the chronic RfD.

EPA expanded the discussion in Section 5.1.3 to clarify the reasoning.

One reviewer wanted a discussion of studies of habitual abuse of chloral hydrate and the consequences of drug withdrawal after habituation. This reviewer also wanted a more extensive discussion of the mechanism of depression of the central nervous system.

EPA believes that it is not necessary to include this information in the Toxicological Review. A discussion of these topics is beyond the scope of the Toxicological Review, which, as stated in the Foreword, is designed to provide support for the hazard identification and exposure-response assessment on IRIS.

One reviewer requested inclusion of information on the organoleptic properties of chloral hydrate in drinking water and the concentration at which adverse taste and odor become a problem.

EPA agrees that including this information would be useful. However, such information was not located in the literature.

One reviewer wanted more discussion of the consequences for humans of the bioaccumulation of trichloroacetic acid.

EPA acknowledges that because trichloroacetic acid binds fairly strongly to human serum proteins, its elimination is slow and it is the only metabolite of chloral hydrate that accumulates with repeated exposure. When exposure to chloral hydrate is at or below the RfD, the concentration of trichloroacetic acid in humans is below the concentration that will cause adverse biological effects.

One reviewer suggested that the LOAEL should be based on the 500 mg/day dose rather than the 750 mg/day dose.

EPA prefers to use the average exposure, rather than the minimum exposure, when deriving a LOAEL. There is also some anecdotal information that chloral hydrate is not effective in a large number of patients when the minimum dose is administered.

APPENDIX B. TOXICOKINETICS OF CHLORAL HYDRATE

This toxicokinetic analysis is used to estimate the steady state concentrations of trichloroacetic acid (TCA) and trichloroethanol (TCEOH) in mice and humans using a one-compartment model, assuming that absorption of chloral hydrate (CH) from the gastrointestinal tract and its metabolism in the blood is very rapid compared to the rate of elimination of TCA and TCEOH. This assumption is supported by the data of Beland et al. (1998) in mice and Breimer (1977) and Zimmermann (1998) in humans.

Beland et al. (1998) indicated that 15% of the dose of chloral hydrate is converted directly to TCA and 77% is converted to TCEOH. In humans Allen and Fisher (1993) estimated that 8% of a dose of chloral hydrate is converted directly to TCA and 92% is converted to TCEOH. Additional TCA is formed from TCEOH. The total TCA formed in humans is approximately 35% of the dose of chloral hydrate.

Estimation of TCA in mice at steady state at the clinically recommended dose for humans

$[TCA]_{ss\text{-}blood}$ = PK_o/VK_{el} = 2.5 mg/L

$[TCA]_{ss\text{-}liver}$ = $[TCA]_{ss\text{-}blood} \times PC$ = 3.0 mg/L

where:

- P = proportion of CH converted to TCA = 0.15 (Beland et al; 1998)
- K_o = dosing rate for CH = 10.7 mg/kg-day, equivalent to 0.446 mg/kg-hr
- V = volume of distribution = 0.321 L/kg (Beland et al; 1998)
- K_{el} = first-order elimination constant for TCA = 0.0819/hr (Beland et al; 1998)
- PC = liver-blood partition coefficient = 1.18 (Abbas and Fisher, 1997)

Estimation of TCA in humans at steady state at the clinically recommended dose

$[TCA]_{ss\text{-}blood}$ = PK_o/VK_{el} = 55 mg/L

$[TCA]_{ss\text{-}liver}$ = $[TCA]_{ss\text{-}blood} \times PC$ = 36 mg/L

where:

- P = proportion of CH converted to TCA = 0.35 (Allen and Fisher, 1993)
- K_o = dosing rate for CH = 10.7 mg/kg-day, equivalent to 0.446 mg/kg-hr
- V = volume of distribution = 0.102 L/kg (Allen and Fisher, 1993)
- K_{el} = first-order elimination constant for TCA = 0.028/hr (Allen and Fisher, 1993)
- PC = liver-blood partition coefficient = 0.66 (Fisher et al; 1998)

Estimation of TCA in humans at steady state at the reference dose

$[TCA]_{ss\text{-}blood}$ = PK_o/VK_{el} = 1.8 mg/L

$[TCA]_{ss\text{-}liver}$ = $[TCA]_{ss\text{-}blood} \times PC$ = 1.2 mg/L

where:

P	=	proportion of CH converted to TCA = 0.35 (Allen and Fisher, 1993)
K_o	=	dosing rate for CH = 0.1 mg/kg-day, equivalent to 0.004 mg/kg-hr
V	=	volume of distribution = 0.102 L/kg (Allen and Fisher, 1993)
K_{el}	=	first-order elimination constant for TCA = 0.0078/hr (Allen and Fisher, 1993)
PC	=	liver-blood partition coefficient = 0.66 (Fisher et al; 1998)

Estimation of TCEOH in mice at steady state at 166 mg/kg-day

$[TCEOH]_{ss\text{-}blood}$ = PK_o/VK_{el} = 0.58 mg/L

where:

P	=	proportion of CH converted to TCEOH = 0.77 (Beland et al; 1998)
K_o	=	dosing rate for CH = 166 mg/kg-day, equivalent to 6.917 mg/kg-hr
V	=	volume of distribution = 1 L/kg (cited in Beland et al; 1998)
K_{el}	=	first-order elimination constant for TCEOH = 9.24/hr (Beland et al; 1998)

Chloral hydrate at 160 mg/kg-day was the highest exposure used in the 90-day neurobehavioral study by Kallman et al. (1984); chloral hydrate at 166 mg/kg-day was the highest exposure used in the 104-week bioassay of Daniel et al. (1992a). These exposures are a NOAEL for sedation in mice.

Estimation of TCEOH in humans at steady state at the clinically recommended dose

$[TCEOH]_{ss\text{-}blood}$ = PK_o/VK_{el} = 5.4 mg/L

where:

P	=	proportion of CH converted to TCEOH = 0.92 (Allen and Fisher, 1993)
K_o	=	dosing rate for CH = 10.7 mg/kg-day, equivalent to 0.446 mg/kg-hr
V	=	volume of distribution = 0.87 L/kg (Fisher et al; 1998)
K_{el}	=	first-order elimination constant for TCEOH = 0.087/hr (Breimer, 1977)

Estimation of TCEOH in adult humans at steady state at the reference dose

$[TCEOH]_{ss\text{-}blood}$ = PK_o/VK_{el} = 0.049 mg/L

where:

P	=	proportion of CH converted to TCEOH = 0.92 (Allen and Fisher, 1993)
K_o	=	dosing rate for CH = 0.1 mg/kg-day, equivalent to 0.004 mg/kg-hr
V	=	volume of distribution = 0.87 L/kg (Fisher et al; 1998)
K_{el}	=	first-order elimination constant for TCEOH = 0.087/hr (Breimer, 1977)

Estimation of TCEOH in preterm infants at steady state at the reference dose

$[TCEOH]_{ss\text{-}blood}$ = PK_o/VK_{el} = 0.25 mg/L

where:

P	=	proportion of CH converted to TCEOH = 0.92 (Allen and Fisher, 1993)
K_o	=	dosing rate for CH = 0.1 mg/kg-day, equivalent to 0.004 mg/kg-hr
V	=	volume of distribution = 0.87 L/kg (Fisher et al; 1998)
K_{el}	=	first-order elimination constant for TCEOH = 0.017/hr (Mayers et al; 1991)

Estimation of TCEOH in full term infants at steady state at the reference dose

$[TCEOH]_{ss\text{-}blood}$ = PK_o/VK_{el} = 0.17 mg/L

where:

P	=	proportion of CH converted to TCEOH = 0.92 (Allen and Fisher, 1993)
K_o	=	dosing rate for CH = 0.1 mg/kg-day, equivalent to 0.004 mg/kg-hr
V	=	volume of distribution = 0.87 L/kg (Fisher et al; 1998)
K_{el}	=	first-order elimination constant for TCEOH = 0.025/hr (Mayers et al; 1991)

Figure 1. Metabolism of Chloral Hydrate

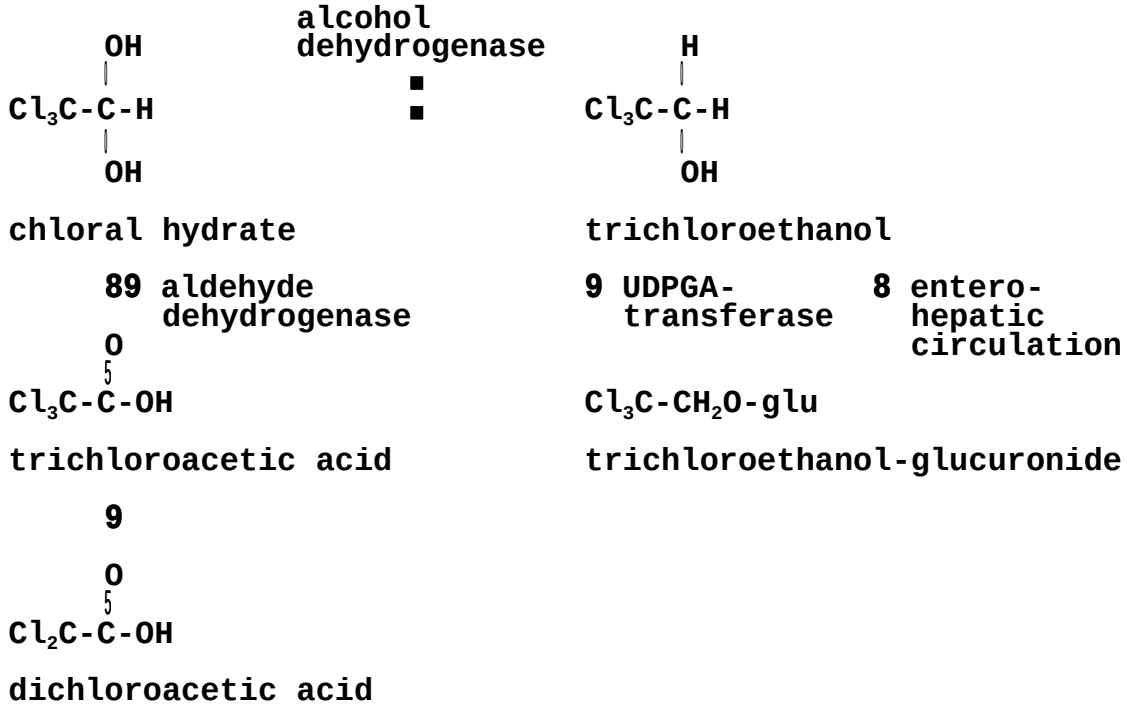

Table 1. Genetic and related effects of chloral hydrate and its metabolites

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
Chloral hydrate				
S. typhimurium, TA 100, reverse mutation	!	!	2,500	Waskell, 1978
S. typhimurium, TA 100, reverse mutation	0	+	2,000	Bruce and Heddle, 1979
S. typhimurium, TA 100, reverse mutation	+	+	500	Haworth et al., 1983
S. typhimurium, TA 100, reverse mutation	!	!	1,850	Leuschner and Leuschner, 1991
S. typhimurium, TA 100, reverse mutation	+	!	300/750	Giller et al., 1995
S. typhimurium, TA 100, reverse mutation	+	+	2,000	Ni et al., 1994
S. typhimurium, TA 104, reverse mutation	+	+	2,000	Ni et al., 1994
S. typhimurium, TA 98, reverse mutation	0	+	2,000	Bruce and Heddle, 1979
S. typhimurium, TA 98, reverse mutation	!	!	5,000	Waskell, 1978
S. typhimurium, TA 98, reverse mutation	!	!	5,000	Haworth et al., 1983
S. typhimurium, TA 98, reverse mutation	!	!	1,850	Leuschner and Leuschner, 1991
S. typhimurium, TA 1535, reverse mutation	!	!	5,000	Haworth et al., 1983
S. typhimurium, TA 1537, reverse mutation	!	!	5,000	Haworth et al., 1983

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
S. typhimurium, TA 1537, reverse mutation	!	!	1,850	Leuschner and Leuschner, 1991
S. typhimurium, TA 1538, reverse mutation	!	!	1,850	Leuschner and Leuschner, 1991
S. cerevisiae D7, reverse mutation	!	!	3,300	Bronzetti et al., 1984
S. cerevisiae D7, gene conversion	!	(+)	2,500	Bronzetti et al., 1984
Gene mutation (small colony), mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	+		1,000	Harrington-Brock et al., 1998
A. nidulans, diploid stain, mitotic crossing over	!	0	1,650	Crebilli et al., 1985
A. nidulans, diploid stain, mitotic crossing over	!	0	6,600	Käfer, 1986
A. nidulans, diploid stain, mitotic crossing over	!	0	1,000	Kappas, 1989
A. nidulans, diploid stain, mitotic crossing over	!	0	990	Crebilli et al., 1985
A. nidulans, diploid stain, haploids and nondisjunctional diploids	+	0	825	Crebilli et al., 1985
A. nidulans, diploid stain, aneuploidy	+	0	825	Käfer, 1986
A. nidulans, haploid conidia, aneuploidy and polyploidy	+	0	825	Käfer, 1986
A. nidulans, diploid stain, nondisjunctional mitotic segregants	+	0	450	Kappas, 1986

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
A. nidulans, diploid stain, haploids and nondisjunctional diploids	+	0	660	Crebilli et al., 1991
A. nidulans, haploid stain, hyperploidy	+	0	2,640	Crebilli et al., 1991
S. cerevisiae, meiotic recombination	?	0	3,300	Sora and Agostini-Carbone, 1987
S. cerevisiae, disomy in meiosis	+	0	2,500	Sora and Agostini-Carbone, 1987
S. cerevisiae, diploids in meiosis	+	0	3,300	Sora and Agostini-Carbone, 1987
S. cerevisiae, mitotic chromosomal malsegregation	+	0	1,000	Albertini, 1990
S. cerevisiae, monosomy	+	0	1,000	Parry et al., 1990
Spring wheat, chromosomal loss or gain	!	0	5,000	Sandhu et al., 1991
D. melanogaster, somatic mutation wing spot test	+		830	Patnaik et al., 1992
D. melanogaster, somatic mutation wing spot test	+		825	Zordan et al., 1994
D. melanogaster, sex-linked recessive lethal mutation	+		1,660	Patnaik et al., 1992
DNA-protein cross-links, rat liver nuclei in vitro	!	0	41,250	Keller and Heck, 1988
DNA single-strand breaks (alkaline unwinding), rat primary hepatocytes in vitro	!	0	1,650	Chang et al., 1992
DNA repair, *E. coli* PQ37, SOS chromotest	!	!	10,000	Giller et al., 1995

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
Kinetochore-positive micronuclei, Chinese hamster C1-1 cells, in vitro, with antikinetochore antibodies	+	0	165	Degrassi and Tanzarella, 1988
Kinetochore-negative micronuclei, Chinese hamster C1-1 cells, in vitro, with antikinetochore antibodies	!	0	250	Degrassi and Tanzarella, 1988
Kinetochore-positive micronuclei, Chinese hamster LUC2 cells in vitro	+	0	400	Parry et al., 1990
Kinetochore-positive micronuclei, Chinese hamster LUC2 cells in vitro	+	0	400	Lynch and Parry, 1993
Inhibition of intercellular communication, $B6C3F_1$ mouse hepatocytes in vitro	!	0	83	Klaunig et al., 1989
Inhibition of intercellular communication, F344 rat hepatocytes in vitro	!	0	83	Klaunig et al., 1989
Chromosomal aberrations, Chinese hamster CHED cells in vitro	+	0	20	Furnus et al., 1990
Aneuploidy, Chinese hamster CHED cells in vitro	+	0	10	Furnus et al., 1990
Aneuploidy, primary Chinese hamster embryonic cells in vitro	+	0	250	Natarajan et al., 1993
Aneuploidy, Chinese hamster LUC2 p4 cells in vitro	+	0	250	Warr et al., 1993

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
Tetraploidy and endoreplication, Chinese hamster LUC2 p4 cells in vitro	+	0	500	Warr et al., 1993
Apolar mitosis, *Haemanthus katherinae* endosperm in vitro	+	0	200	Molè-Bajer, 1969
Inhibition of spindle elongation, PtK2 kangaroo rat kidney epithelial cells in vitro	+	0	1,000	Lee et al., 1987
Inhibition of chromosome to pole movement, PtK2 kangaroo rat kidney epithelial cells in vitro	+	0	1,000	Lee et al., 1987
Breakdown of mitotic microtubuli, PtK2 kangaroo rat kidney epithelial cells in vitro	+	0	1,000	Lee et al., 1987
Multipolar mitotic spindles, Chinese hamster DON:Wg3h cells in vitro	+	0	500	Parry et al., 1990
Chromosomal dislocation from mitotic spindle, Chinese hamster DON:Wg3h cells in vitro	+	0	500	Parry et al., 1990
Lacking mitotic spindle, Chinese hamster DON:Wg3h cells in vitro	+	0	250	Parry et al., 1990
Metaphase defects, lacking mitotic spindle, Chinese hamster LUC1 cells in vitro	+	0	50	Parry et al., 1990

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
Multipolar mitotic spindles, Chinese hamster DON:Wg3h cells in vitro	+	0	50	Warr et al., 1993
Chromosomal dislocation from mitotic spindle, Chinese hamster DON:Wg3h cells in vitro	+	0	500	Warr et al., 1993
DNA single-strand breaks (alkaline unwinding), human lymphoblastoid CCRF-CEM cells in vitro	!	0	1,650	Chang et al., 1992
Sister chromatid exchange, human lymphocytes in vitro	(+)	0	54	Gu et al., 1981
Micronucleus induction, isolated human lymphocytes in vitro	+	!	1,500	Vian et al., 1995
Micronucleus induction, human lymphocytes in whole blood in vitro	+	0	100	Migliore and Nieri, 1990
Micronucleus induction, human lymphocytes in vitro	(+)	0	100	Ferguson et al., 1993
Micronucleus induction, human lymphocytes in vitro	+	0	100	Van Hummelen and Kirsch-Volders, 1992
Micronuclei, Chinese hamster V79 cells in vitro	+	0	316	Seelbach et al., 1993
Micronucleus induction, newt (*Pleurodeles waltl*) larvae, peripheral erythrocytes in vivo	+		200	Giller et al., 1995
Micronucleus induction, mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	!		1,250	Harrington-Brock et al., 1998

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	Result[a] With	Dose[b] (LED/HID)	Reference
Chromosomal aberrations, mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	+		1,250	Harrington-Brock et al., 1998
Kinetochore-positive micronuclei, human diploid LEO fibroblasts in vitro	+	0	120	Bonatti et al., 1992
Aneuploidy, human lymphocytes in vitro	+	0	250	Vagnarelli et al., 1990
Aneuploidy, human lymphocytes in vitro	+	0	50	Sbrana et al., 1993
Polyploidy, human lymphocytes in vitro	+	0	137	Sbrana et al., 1993
C-Mitosis, human lymphocytes in vitro	+	0	75	Sbrana et al., 1993
Polyploidy, mice, outbred MF-1, oocytes in vitro	+		50	Eichenlaub-Ritter and Betzendahl, 1995
Polyploidy, mice, outbred MF-1, oocytes in vitro	+		125	Eichenlaub-Ritter et al., 1996
Host-mediated assay, *S. cerevisiae* D7 recovered from CD-1 mouse lungs	(+)		500 po × 1	Bronzetti et al., 1984
DNA single-strand breaks (alkaline unwinding), rat liver in vivo	+		300 po × 1	Nelson and Bull, 1988
DNA single-strand breaks (alkaline unwinding), mouse liver in vivo	+		100 po × 1	Nelson and Bull, 1988
DNA single-strand breaks (alkaline unwinding), male Fischer 344 rat liver in vivo	!		1,650 po × 1	Chang et al., 1992

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	Result[a] With	Dose[b] (LED/HID)	Reference
DNA single-strand breaks (alkaline unwinding), male B6C3F_1 mouse liver in vivo	!		825 po × 1	Chang et al., 1992
Chromosomal aberrations, (c57Bl/Cne×C3H/Cne)F1 mouse secondary spermatocytes (staminal gonia-pachytene treated)	+		82.7 ip × 1	Russo et al., 1984
Chromosomal aberrations (translocations, breaks, and fragments) (C57Bl/Cne×C3H/Cne)F1 mouse primary and secondary spermatocytes (from differentiating spermatogonia-pachytene stages treated)	!		413 ip × 1	Liang and Pacchierotti, 1988
Chromosomal aberrations, male and female (102/E1×C3H/E1)F1 mouse bone marrow cells in vivo	!		500 ip × 1	Xu and Adler, 1990
Chromosomal aberrations, rat bone marrow cells in vivo	!		1,000 po × 1	Leuschner and Leuschner, 1991
Chromosomal aberrations, BALB/c mouse spermatogonia treated, spermatogonia observed in vivo	!		83 ip × 1	Russo and Levis, 1992a
Chromosomal aberrations, ICR mouse oocytes treated in vivo	!		600 ip × 1	Mailhes et al., 1993
Micronuclei, female mice (C57Bl6×C3H/He)F1, bone marrow erythrocytes	!		2,500 ip × 5	Bruce and Heddle, 1979

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	Result[a] With	Dose[b] (LED/HID)	Reference
Micronuclei, male and female NMRI mice, bone marrow erythrocytes in vivo	!		500 ip × 1	Leuschner and Leuschner, 1991
Micronuclei, mouse spermatids in vivo (preleptotene spermatocytes treated)	!		83 ip × 1	Russo and Levis, 1992b
Micronuclei, male BALB/c mouse bone marrow erythrocytes in vivo	+		83 ip × 1	Russo and Levis, 1992a
Micronuclei (kinetochore-positive and -negative) male BALB/c mouse bone marrow erythrocytes in vivo	+		200 ip × 1	Russo et al., 1992
Micronuclei, BALB/c mouse early spermatids in vivo (diakinesis/metaphase I and metaphase II stages treated)	+		83 ip × 1	Russo and Levis, 1992a
Micronuclei, male (C57Bl/Nce×C3H/Cne)F1 mouse bone marrow erythrocytes in vivo	!		400 ip × 1	Leopardi et al., 1993
Micronuclei, mouse spermatids in vivo (spermatogonial stem cells and preleptotene spermatocytes treated)	+		41 ip × 1	Allen et al., 1994
Micronuclei, mice (102/E1×C3H/E1)F1, polychromatic bone marrow erythrocytes	!		600 ip × 1	Adler et al., 1991

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	Result[a] With	Dose[b] (LED/HID)	Reference
Micronuclei, mice (102/E1×C3H/E1)F1, polychromatic bone marrow erythrocytes	!		200 ip × 1	Grawe et al., 1997
Micronuclei, mice ($B6C3F_1$), spermatids in vivo (spermatogonial stem cells treated)	+		82.7 ip × 1	Nutley et al., 1996
Micronuclei, mice ($B6C3F_1$), spermatids in vivo (preleptotene and diakinesis spermatids treated)	!		413.5 ip × 1	Nutley et al., 1996
Aneuploidy, (C57Bl/Cne×C3H/Cne)F1, mouse secondary spermatocytes in vivo	+		82.7 ip × 1	Russo et al., 1984
Aneuploidy, (C57Bl/Cne×C3H/Cne)F1, mouse secondary spermatocytes (from differentiating spermatogonia-pachytene stages treated)	(+)		165 ip × 1	Liang and Pacchierotti, 1988
Aneuploidy, ICR mouse, metaphase II oocytes in vivo	!		200 ip × 1	Maihles et al., 1994
Aneuploidy (hyperploidy). ICR mouse metaphase II oocytes in vivo	!		600 ip × 1	Maihles et al., 1994
Polyploidy, male and female 102/E1×C3H/E1)F1 mouse bone marrow cells in vivo	!		600 ip × 1	Xu and Adler, 1990

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
Aneuploidy, (102/E1×C3H/Cne)F1 mouse secondary spermatocytes in vivo	+		200 ip × 1	Miller and Adler, 1992
Aneuploidy, male (C57Bl/Nce×C3H/Cne)F1 mouse bone marrow in vivo	+		400 ip × 1	Leopardi et al., 1993
Aneuploidy, (C57Bl/Nce×C3H/Cne)F1 mouse secondary spermatocytes in vivo	!		400 ip × 1	Leopardi et al., 1993
Aneuploidy, male mice (CD-1), bone marrow erythrocytes	+		200 ip × 1	Gudi et al., 1992
Aneuploidy (micronuclei), male mice (CD-1), bone marrow erythrocytes	+		200 ip × 1	Marrazzini et al., 1994
Binding to DNA, male $B6C3F_1$ mouse liver in vivo	!		800 ip × 1	Keller and Heck, 1988
Gonosomal and autosomal univalents (C57Bl/Cne×C3h/Cne)F1 mouse primary spermatocytes (from differentiating spermatogonia-pachytene stages treated)	!		413 ip × 1	Liang and Pacchierotti, 1988
Porcine brain tubulin assembly inhibition in vitro	+	0	9,900	Brunner et al., 1991
Porcine brain tubulin assembly inhibition in vitro	+	0	40	Brunner et al., 1991
Porcine brain tubulin assembly inhibition in vitro	(+)	0	165	Wallin and Hartley-Asp, 1993

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
Centriole migration block, Chinese hamster cells clone 237 in vitro	+	0	1,000	Alov and Lyubskii, 1974
Cell transformation, Syrian hamster embryo	+		350, 1 day 1, 7 day	Gibson et al., 1995
Trichloroethanol				
8 Prophage induction, *E. coli* WP2	!	!	155,000	DeMarini et al., 1994
S. typhimurium, TA 100, reverse mutation	!	!	0.5 vapor	DeMarini et al., 1994
Spindle aberrations, mice, outbred MF-1, oocytes in vitro	+		125	Eichenlaub-Ritter et al., 1996
Trichloroacetic acid				
8 prophage induction, *E. coli* WP2s	!	!	10,000	DeMarini et al., 1994
S. typhimurium TA100, reverse mutation	!	!	225	Waskell, 1978
S. typhimurium TA100, reverse mutation	!	!	2,000	Nestmann et al., 1980
S. typhimurium TA100, reverse mutation	!	!	4	DeMarini et al., 1994
S. typhimurium TA1535, reverse mutation	!	!	2,000	Nestmann et al., 1980
S. typhimurium TA1537, reverse mutation	!	!	1,000	Nestmann et al., 1980
S. typhimurium TA1538, reverse mutation	!	!	1,000	Nestmann et al., 1980
S. typhimurium TA98, reverse mutation	!	!	225	Waskell, 1978

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	Result[a] With	Dose[b] (LED/HID)	Reference
S. typhimurium TA98, reverse mutation	!	!	1,000	Nestmann et al., 1980
Gene mutation (small and large colony), mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	!	+	2,250	Harrington-Brock et al., 1998
Chromosomal aberrations, human lymphocytes in vitro	!	!	5,000	Mackay et al., 1995
DNA strand breaks, B6C3F$_1$ mouse hepatocytes in vitro	!	0	1,630	Chang et al., 1992
DNA strand breaks, Fischer 344 rat hepatocytes in vitro	!	0	1,630	Chang et al., 1992
DNA strand breaks, human CCRF-CEM cells in vitro	!	0	1,630	Chang et al., 1992
DNA strand breaks, B6C3F$_1$ mouse hepatic cells in vivo	+		1 po × 1	Nelson and Bull, 1988
DNA strand breaks, B6C3F$_1$ mouse hepatic cells in vivo	+		500 po × 1	Nelson et al., 1989
DNA strand breaks, B6C3F$_1$ mouse hepatic cells in vivo	!		500 po × 10	Nelson et al., 1989
DNA strand breaks, B6C3F$_1$ mouse hepatic cells and epithelial cells from stomach and duodenum in vivo	!		1,630 po × 1	Chang et al., 1992
DNA strand breaks, Sprague-Dawley rat hepatic cells in vivo	+		100 po × 1	Nelson and Bull, 1988
DNA strand breaks, Fischer 344 rat hepatocyte cells in vivo	!		1,630 po × 1	Chang et al., 1992

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	With	Dose[b] (LED/HID)	Reference
Micronucleus induction, Swiss mice in vivo	+		125 ip × 1	Bhunya and Behera, 1987
Micronucleus induction, C57Bl/6JfB110/Alpk female mice	!		1,300 ip × 2	Mackay et al., 1995
Micronucleus induction, C57Bl/6JfBl10/Alpk male mice	!		1,080 ip × 1	Mackay et al., 1995
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	+		125 ip × 1	Bhunya and Behera, 1987
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	+		100 ip × 5	Bhunya and Behera, 1987
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	+		500 po × 1	Bhunya and Behera, 1987
Chromosomal aberrations, chicken bone marrow cells in vivo	+		200 ip × 1 400 po × 1	Bhunya and Jena, 1996
Dichloroacetic acid				
8 prophage induction, *E. coli* WP2s	!	+	2,500	DeMarini et al., 1994
S. typhimurium, DNA repair-deficient TS24	!	!	31,000	Waskell, 1978
S. typhimurium, DNA repair-deficient TA2322	!	!	31,000	Waskell, 1978
S. typhimurium, DNA repair-deficient TA1950	!	!	31,000	Waskell, 1978
S. typhimurium, TA100, reverse mutation	(+)	(+)	1	DeMarini et al., 1994

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	Result[a] With	Dose[b] (LED/HID)	Reference
S. typhimurium, TA98, reverse mutation	(+)	(+)	5	Herbert et al., 1980
Gene mutation (small colony), mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	+	0	300	Harrington-Brock et al., 1998
Chromosomal aberrations, mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	+	0	600	Harrington-Brock et al., 1998
Micronuclei, mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	!	0	800	Harrington-Brock et al., 1998
aneuploidy, mouse lymphoma cells (L5178Y/TK$^{\pm}$-3.7.2.C)	!	0	800	Harrington-Brock et al., 1998
DNA strand breaks, B6C3F$_1$ mouse hepatocytes in vitro	!	0	2,580	Chang et al., 1992
DNA strand breaks, Fischer 344 rat hepatocytes in vitro	!	0	1,290	Chang et al., 1992
DNA strand breaks, human CCRF-CEM cells in vitro	!	0	1,290	Chang et al., 1992
DNA strand breaks, B6C3F$_1$ mouse hepatic cells in vivo	+		13 po × 1	Nelson and Bull, 1988
DNA strand breaks, B6C3F$_1$ mouse hepatic cells in vivo	+		10 po × 1	Nelson et al., 1989
DNA strand breaks, B6C3F$_1$ mouse hepatic cells in vivo	!		1,290 po × 1	Chang et al., 1992
DNA strand breaks, B6C3F$_1$ mouse spleenocytes in vivo	!		1,290 po × 1	Chang et al., 1992

Table 1. Genetic and related effects of chloral hydrate and its metabolites (continued)

Test system	Result[a] Without	Result[a] With	Dose[b] (LED/HID)	Reference
DNA strand breaks, $B6C3F_1$ mouse epithelial cells from stomach and duodenum in vivo	!		1,290 po × 1	Chang et al., 1992
DNA strand breaks, $B6C3F_1$ mouse hepatic cells in vivo	!		830 po × 7-14 d	Chang et al., 1992
DNA strand breaks, Sprague-Dawley rat hepatic cells in vivo	+		30 po × 1	Nelson and Bull, 1988
DNA strand breaks, Fischer 344 rat hepatic cells in vivo	!		645 po × 1	Chang et al., 1992
DNA strand breaks, Fischer 344 rat hepatic cells in vivo	!		250 po × 30 weeks	Chang et al., 1992
Gene mutation, transgenic $B6C3F_1$ mouse in vivo	+		160 po × 60 weeks	Leavitt et al., 1997
Micronucleus induction, mouse polychromatic erythrocytes	+		160 po × 9	Fuscoe et al., 1992

[a] Without: without an exogenous metabolic activation system; With: with an exogenous metabolic activation system; +: conclusion considered to be positive; (+): considered to be weakly positive in an inadequate study; !: considered to be negative; ?: considered to be inconclusive (variable responses in several experiments within an inadequate study); 0: not tested.

[b] LED, lowest effective dose; HID, highest ineffective dose; in vitro tests, µg/ml; in vivo tests, mg/kg bw; ip, interperitoneal; po, orally.

Table 2. Summary of nonneoplastic effects

Species	Duration	Endpoint	NOAEL mg/kg-day	LOAEL mg/kg-day	Reference
Human	1 day, 3 doses	CNS depression, GI irritation	—	10.7	Goodman and Gilman, 1985
Rat	90 days	Liver necrosis and increase in serum enzymes	96	168	Daniel et al., 1992b
Rat	104 weeks	—	162.6	—	George et al., 2000
Rat	124 weeks	Liver hypertrophy	45	135	Leuschner and Beuscher, 1998
Rat	52 weeks	Sperm motility	55	188	Klinefelter et al., 1995
Rat	gd 1-22	Development	151	—	Johnson et al., 1998
Mouse	14 days	Increased liver weight	14.4	144	Sanders et al., 1982
Mouse	90 days	Increased liver weight	16	160	Sanders et al., 1982
Mouse	104 weeks	Increased liver weight and necrosis	—	166[a]	Daniel et al., 1992a
Mouse	104 weeks	—	146.6[b]	—	George et al., 2000
Mouse	104 weeks	—	71.4[c]	—	NTP, 2000a,b

Table 2. Summary of nonneoplastic effects (continued)

Species	Duration	Endpoint	NOAEL mg/kg-day	LOAEL mg/kg-day	Reference
Mouse	3 weeks pre-breeding and during gestation	Reproduction and development	204.8	-	Kallman et al., 1984
Mouse	Pre-breeding, gestation, and nursing	Passive avoidance learning in pups	21.3	204.8	Kallman et al., 1984
Mouse	1 day	Ataxia	-	50	Kallman et al., 1984
Mouse	14 days	Neuro-behavior	144	-	Kallman et al., 1984
Mouse	90 days	Neuro-behavior	160	-	Kallman et al., 1984
Mouse	14 days	Immuno-toxicity	144	-	Kauffmann et al., 1982
Mouse	90 days	Humoral immunity	16	160	Kauffmann et al., 1982

[a] Increased incidence of hepatocellular adenomas and carcinomas at 166 mg/kg-day.
[b] Increased prevalence of hyperplasia and hepatocellular adenomas or carcinomas at 13.5, 65, and 146.6 mg/kg-day.
[c] Increased incidence of adenoma in pituitary gland par distalis in females and hepatocellular carcinoma in males.